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In vitro evaluation of alginate beads of a diclofenac salt

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Abstract

Alginate beads containing diclofenac hydroxyethylpyrrolidine were formulated with either Eudragit or chitosan in order to achieve an enteric formulation. In all cases, high entrapment efficiencies were obtained. The examination of fractured beads revealed an internal void in the Eudragit–alginate beads. In contrast, a dense homogeneous internal structure was observed in the chitosan–alginate beads due to interpolymeric complex. An interaction between chitosan and drug was also observed. Under conditions mimicking those in the stomach, a small amount of drug was released. The alginate–chitosan beads showed a release behaviour dependent on pH value and alginate–chitosan ratio.

Keywords: Sodium alginate; Chitosan; Eudragit L30D; Interpolymeric-complex; Multiple unit-system; Controlled release

1. Introduction

Diclofenac is a widely used non-steroidal anti-inflammatory drug for the treatment of rheumatoid arthritis, osteoarthritis and ankylosing spondylitis. The molecule is practically water insoluble, but it is readily absorbed from the gastrointestinal tract as the salt form. Chronic oral administration of diclofenac may increase the chance of gastrointestinal (GI) damage including bleeding, ulceration and perforation (Scholer et al., 1986). In order to eliminate these adverse effects, enteric coated products and/or sustained release forms have been developed and commercialised (Lin and Kao, 1991; Vilivalam and Adeyeye, 1994; Torres et al., 1995; Okada et al., 1996).

In recent years, multiparticulate systems have received a considerable interest. The influence of gastric emptying time and intestinal motility on intra and inter-subject variation in the rate and

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the extent of availability can be largely avoided by the use of multiple-unit dosage forms (Folkier and Doelker, 1986; Coupe et al., 1991; Clarke et al., 1993; Hogan and Aulton, 1995). It is accepted that the size of most multiparticulates enables them to pass through the constricted pyloric sphincter so that they are able to distribute themselves along the entire GI tract (Bechgaard and Ladefoged, 1978).

Diclofenac hydroxyethylpyrrolidine is a diclofenac salt that exhibits good biopharmaceutical properties. The purpose of this study is to investigate a multiparticulate enteric preparation of the diclofenac salt, based on alginates. The enteric protection of the preparation is achieved by mixing the alginates with either Eudragit® L30D or chitosan. Eudragit L30D is a polymethylmethacrylate derivate formulated in an aqueous dispersion which is mixed with an aqueous solution of sodium alginate. Chitosan is capable of reacting with alginate to form a polyelectrolyte complex on the basis of their opposite charges (Polk et al., 1994; Li, 1996; Lin et al., 1997).

2. Materials and methods

2.1. Materials

Diclofenac hydroxyethylpyrrolidine (DHEP) was a gift from IBSA (Lugano, Switzerland). Eudragit® L30D was received from Industrias Sintéticas Curtex (Barcelona, España). The following materials were obtained from the indicated suppliers and used as received: sodium alginate (low viscosity; viscosity of 2% solution 25°C, ≈ 250 cps), calcium chloride and Chitosan (Practical grade from crab shell; Sigma, Barcelona, España), di-sodium hydrogen phosphate anhydrous, potassium di-hydrogen phosphate, ethylenediamine tetracetic acid and hydrochloric acid 35% (Panreac Química S.A., Barcelona, España), acetic acid glacial 100% (Merck, Barcelona, España).

2.2. Preparation of beads

Eudragit® L30D/Alginate beads were prepared as follows: an alginate solution of 1.5% w/v was prepared dissolving the sodium alginate in distilled water with agitation. The drug (2.5% w/v) was added to the solution and the resulting solution was then stirred until dissolution was complete. Eudragit® L30D was partially neutralised with 1 N NaOH to obtain a 30% degree of neutralisation. This allows partial dissolution of the polymer. A total of 10 ml of this partially neutralised polymer was added to the alginate–drug solution and dropped, from a hypodermic syringe, into 1.3% w/v $CaCl_2$ solution. The gel beads that formed immediately in the $CaCl_2$ solution were incubated at room temperature (22°C) in this solution for 24 h to ensure complete reaction. After this time, the microspheres were filtered and dried at room temperature for 24 h.

In order to prepare the chitosan/alginate beads, chitosan solutions of 0.1 and 0.2% (w/v) were prepared by adding chitosan to distilled water containing 1% (v/v) acetic acid. The solution was stirred for 1 h. The 1.3% w/v $CaCl_2$ solution was added to the chitosan solution. An alginate/drug solution was added to this solution to form the beads. The beads were incubated and dried under the same conditions as described above.

2.3. Drug loading

2.3.1. Indirect method

Aliquots from the filtered solutions remaining after removal of the beads were assayed spectrophotometrically at 276 nm. The amount of DHEP entrapped in the beads was calculated from the difference between the total amount of DHEP added and the DHEP found in the filtered solution.

2.3.2. Thermal analysis

Differential scanning calorimetry (DSC) analysis was used to characterize the thermal behaviour of the different beads components. This analytical method was carried out on isolated substances, their physical mixtures and empty and loaded beads. DSC thermograms were obtained using an automatic thermal analyzer system (Mettler FP80HT Central Processor and Mettler FP85TA Cell). A data processing system (Mettler FP89HT) was connected to the thermal analyzer. Sealed and

perforated aluminium pans were used in the experiments for all the samples. Temperature calibrations were performed using indium as a standard. An empty pan, sealed in the same way as the sample, was used as a reference. All samples were run at a scanning rate of 10°C/min, from 40–300°C.

2.4. Scanning electron microscopy

Scanning electron microscopy (Philips, XL30) was used to examine the morphology and surface structure of the beads. A thin coat of carbon was applied, under vacuum, to each sample, prior to examination.

2.5. Qualitative element analysis

Energy dispersive X-ray analysis (EDAX) (Philips XL30) was used to identify the main components of the bead formulations. This microanalysis technique permits evaluation in relation to the atomic weight, the elemental composition of the substance under study. The analysis was realized on the surface and inner regions of beads to know the behaviour of sodium and calcium atoms.

2.6. Dissolution studies

Drug release was determined using 240 mg of beads, introduced in hard gelatin capsules, in 1l of dissolution medium at 37 ± 0.5°C and a stirring rate of 50 rpm. In all the studies, the XXIII USP basket apparatus (Turu Grau, model D-6) was used. The dissolution medium was either pH 1.1 HCl 0.1 N, or 7.4 Sørensens phosphate buffer. Samples (2 ml) were withdrawn at specific time intervals and assayed spectrophotometrically at the wavelength of maximum absorbance ($\lambda = 276$ nm). From the absorbance values, the cumulative percent released was determined. Samples of 200 μm particle size were chosen for the study. All the studies were carried out in triplicate.

3. Results and discussion

3.1. Entrapment efficiency

Total DHEP percent entrapment and the surface drug, obtained from the indirect method, in Eudragit® L30D/alginate and chitosan/alginate beads are shown in Table 1. The yield of the formulation containing Eudragit® L30D was only 69.98%, while the best results were obtained by using chitosan (91.26 and 95.99%). This lower percentage of entrapment in the Eudragit L30D/algiante beads can be attributed to the presence of electrostatic repulsion forces between the neutralized acrylic polymer (anionic form) and the diclofenac anion due to their negative charges. On the other hand and as it is indicated in the next section, a possible interaction between the cationic polymer chitosan and DHEP is developed and therefore, the entrapment efficiency is greater when chitosan is used.

3.2. Thermal analysis

In the DSC studies several interactions can be identified:

1. Alginate–Ca: the degradation exotherm of alginate at ≈ 240°C, is absent and at 190°C, an endotherm corresponding to alginate–Ca interaction is observed (Fig. 1).
2. Alginate–Ca–chitosan (blank beads): this thermogram is similar to the above (Fig. 1). The broad endothermic peak around 100°C can be attributable to the polyelectrolyte interaction between chitosan and the alginate as

Table 1
Entrapment efficiency of DHEP in the beads

	Percent entrapment (%) ± S.D.	Drag surface (%) ± S.D.
Eudragit® L30D/ alginate	69.97 ± 0.014	6.23 ± 0.013
0.1% Chitosan/ alginate	95.99 ± 0.005	4.04 ± 0.57
0.2% Chitosan/ alginate	91.27 ± 0.009	6.86 ± 0.007

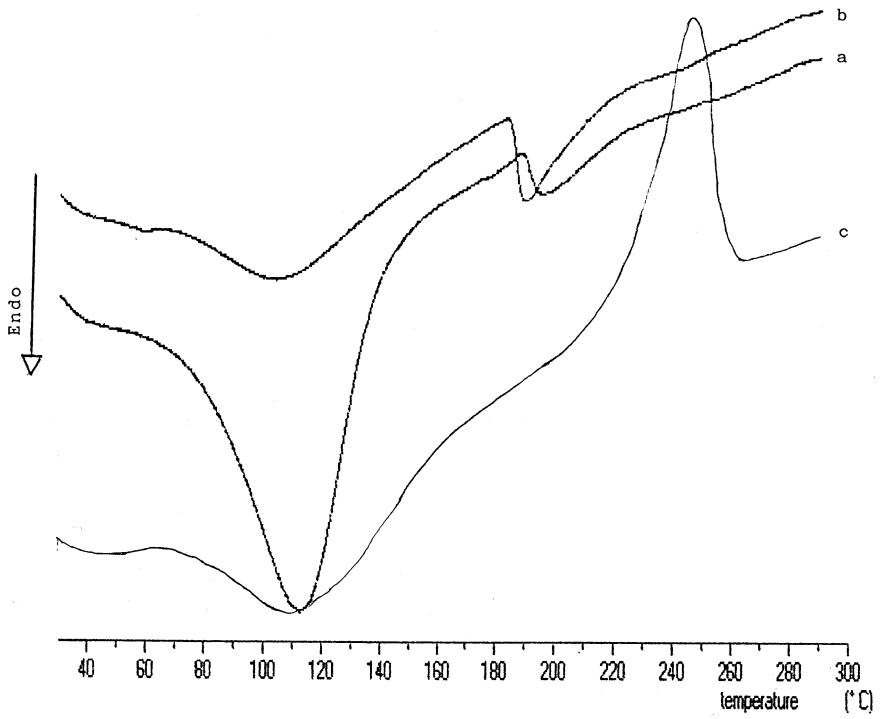

Fig. 1. Thermograms of the samples obtained from: (a) Physical mixture sodium alginate and calcium chloride; (b) blank beads; and (c) sodium alginate.

previously reported (Polk et al., 1994; Li, 1996; Lin et al., 1997).

3. Drug–chitosan: further studies were carried out, in order to investigate a possible interaction between DHEP and chitosan. An aqueous solution of both substances was prepared and maintained with stirring for 7 days at room temperature. The precipitate formed was separated by filtration and analysed using DSC. The thermogram obtained was completely different to that obtained with the drug–chitosan physical mixture (Fig. 2). This result is in agreement with those previously reported by Imai et al. (1991) and Berthold et al. (1996) who postulated that chitosan, a basic polysaccharide, interacts with acidic drugs rather than basic drugs. This implies that the carboxyl group in DHEP and the amino group in Chitosan are important in the bonding process, and the thermogram obtained corresponds to a new structure formed by the complexation of DHEP and chitosan. Additionally, this interaction allows a higher drug entrapment into the beads during their preparation (Table 1). When chitosan is replaced by Eudragit® L30D, an anionic polymer, the negative charges of drug and polymer goes rise to repulsion forces that lead to a lower drug entrapment.

These results suggest that chitosan plays an important role in the formation and stability of the DHEP alginate beads.

3.3. Morphology of the beads

All the beads produced were spherical in form. Scanning electron micrographs show that all the samples maintain this spherical form after drying at room temperature (Fig. 3).

The samples prepared with 0.2% chitosan showed a tendency to form agglomerates. This fact can be attributed to the adhesive properties of chitosan. When the percentage of chitosan was increased to values close to 0.3%, the beads agglomerates permanently $CaCl_2$/chitosan solution and separation was impossible. Similar observa-

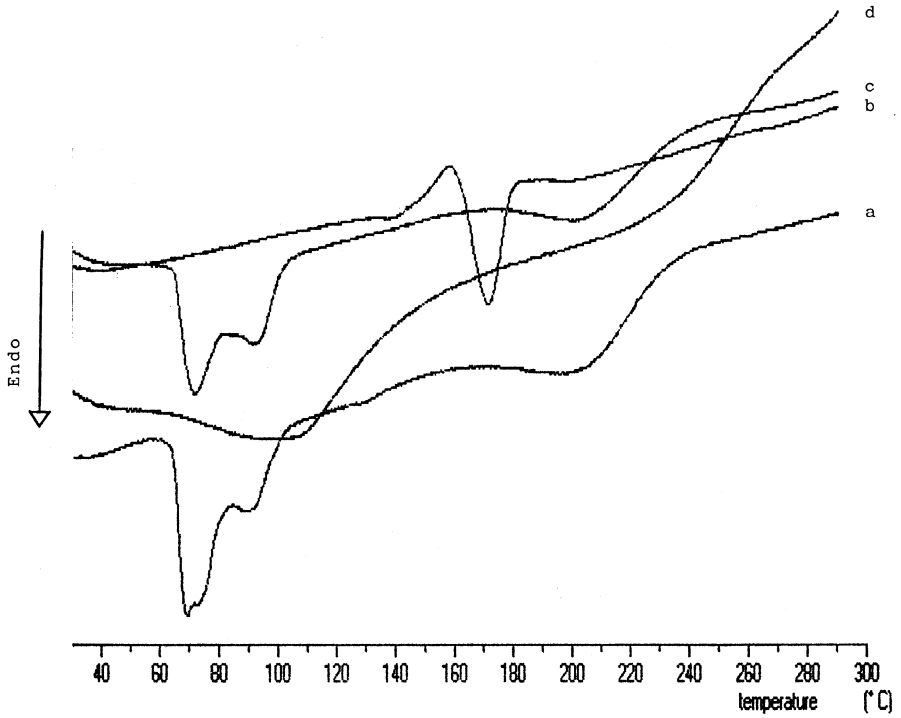

Fig. 2. Thermograms of the samples obtained from: (a) DHEP; (b) chitosan; (c) drug-chitosan physical mixture; and (d) drug-chitosan precipitated.

tions were made by Filipovic-Grcic et al., 1996. These authors reported that a highly concentrated solution of chitosan made the dropping process difficult and microspheres could not readily be formed. For this reason chitosan was always employed at a concentration $< 0.25\%$ (w/v). Higher concentration were not practical because the viscosity was too high.

One noticeable characteristic of the beads surface is its high porosity when the percentage of chitosan increases (Fig. 3c). This fact can be explained on the basis of the high viscosity of the chitosan–calcium chloride solution that prevents the complete interaction between Ca^{+2} and alginate. The non-reacting alginate is deposited on the bead surface during the incubation period. This fact is further clarified by means of EDAX analysis.

The examination of fractured beads (Fig. 4) revealed an internal void in the Eudragit® L/alginate beads (Fig. 4a). In contrast, a dense homogeneous internal structure was observed in the chitosan–alginate beads.

3.4. Elemental analysis by EDAX

The EDAX analysis of the beads was carried out at two different intensities in order to analyse the different elemental bead composition at both the surface and inner regions. In the Eudragit® L/alginate beads, the EDAX analysis shown in Fig. 5, reveals a lack of Na atoms in both, surface and inner. This fact was attributed to the cross-linking reaction between alginate and calcium to form the well know egg-box structure (Smidsrod and Skjak-Braek, 1990). Similar results can be observed in the elemental analysis of 0.1% chitosan/alginate beads (Fig. 6). However, when samples of 0.2% chitosan/alginate beads were studied, a clear peak due to the presence of Na atoms was found in the EDAX analysis (Fig. 7). These results suggest that the presence of a high chitosan concentration in the calcium solution forms a viscous solution and when the alginate solution is dropped in, the reaction between both substances is limited. Thus, the noticeable peak of Na atoms and the lack of

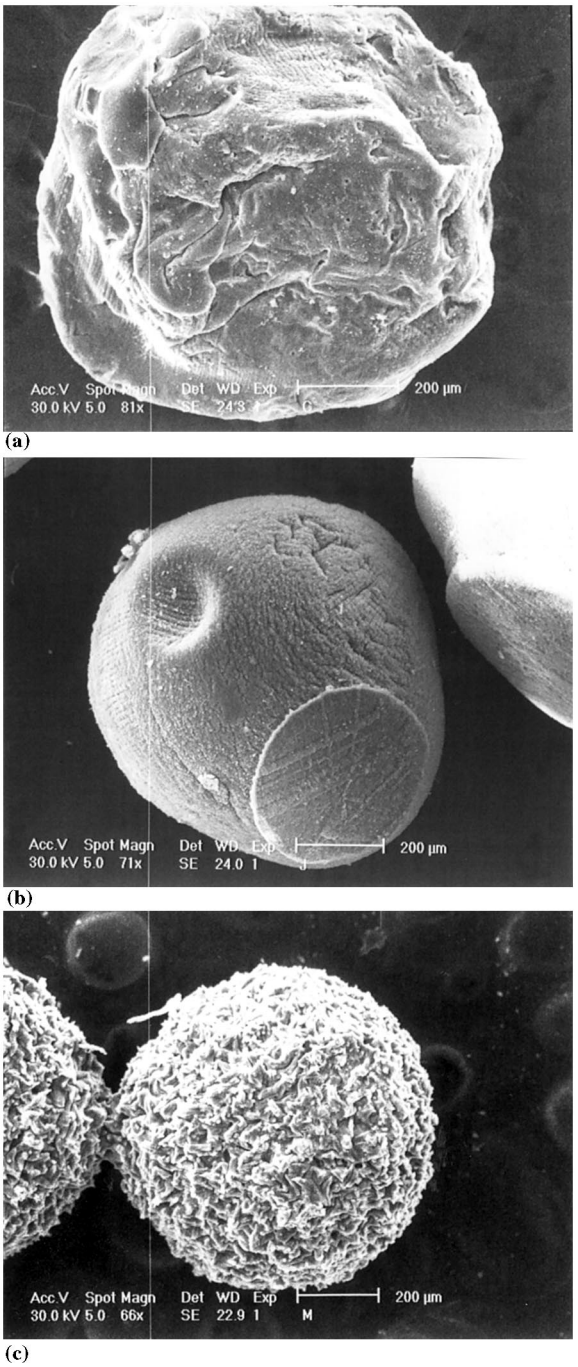

Fig. 3. Microphotographs of the beads obtained from: (a) Eudragit–alginate; (b) chitosan (0.1%)–alginate; and (c) chitosan (0.2%)–alginate.

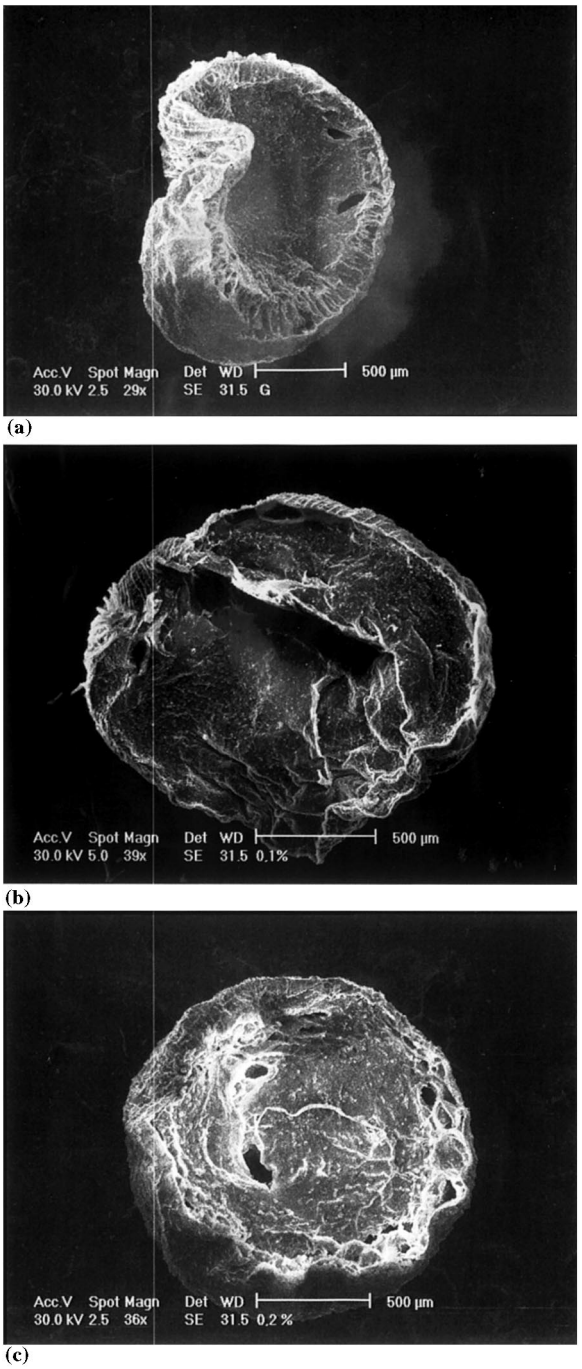

Fig. 4. Microphotographs of the internal beads surface obtained from: (a) Eudragit–alginate; and (b) chitosan (0.1%)–alginate; and (c) chitosan (0.2%)–alginate.

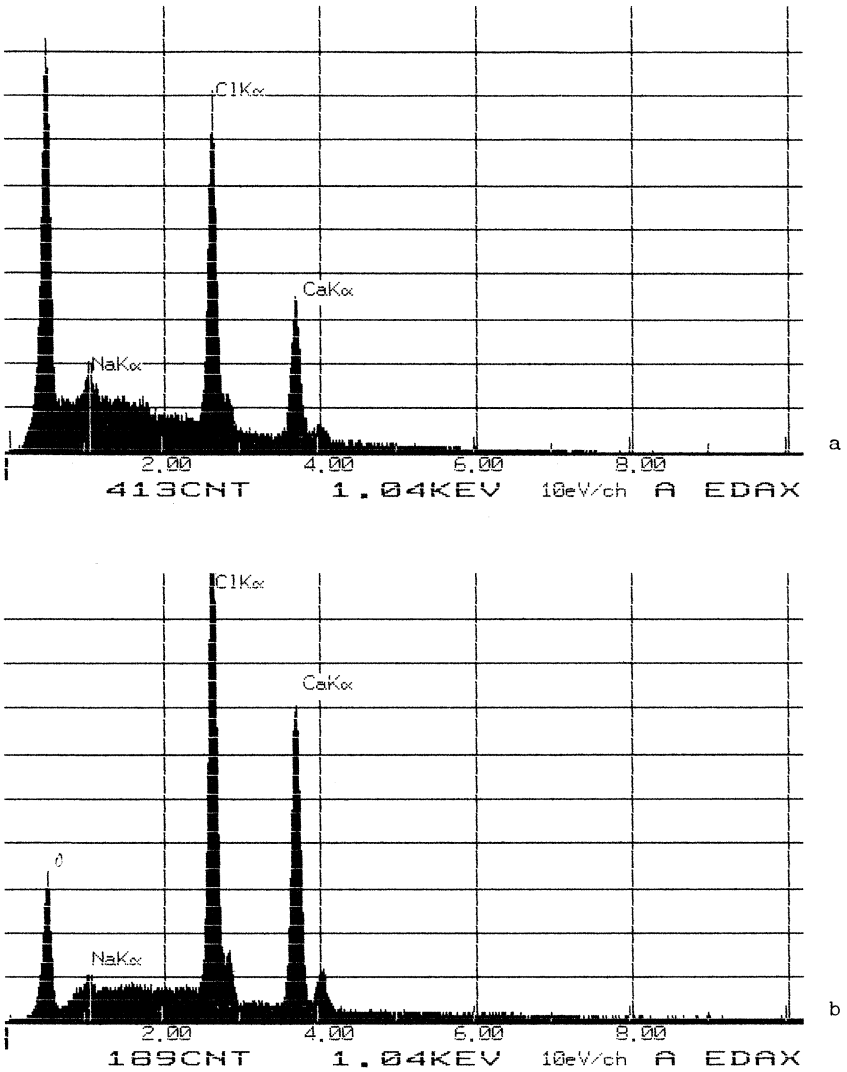

Fig. 5. Microanalysis chart corresponding to the Eudragit–alginate beads: (a) Surface; and (b) inner.

calcium atoms in the beads can be considered as evidence, that part of sodium alginate has not reacted and is deposited on the surface (Fig. 3c).

In all the cases, it was possible to notice a chloride peak corresponding to the atoms present in the DHEP molecule (Figs. 5–7). When the electron intensity was increased, this peak became more evident. This behaviour is a clear indication of the presence of drug molecules in the inner region of the beads indicating a high entrapment efficiency.

3.5. Drug release studies

The drug release profiles from beads are shown in Figs. 8–10. Under conditions mimicking those in the stomach, pH 1.1, only a small amount of drug was released during the test (5 h). This corresponded to the drug deposited on the surface of the beads. Once this drug was removed, the insoluble nature of the pH-dependent polymer, Eudragit® L30D and the controlled release characteristics of the chitosan–alginate interpolymeric complex presented the release of the DHEP.

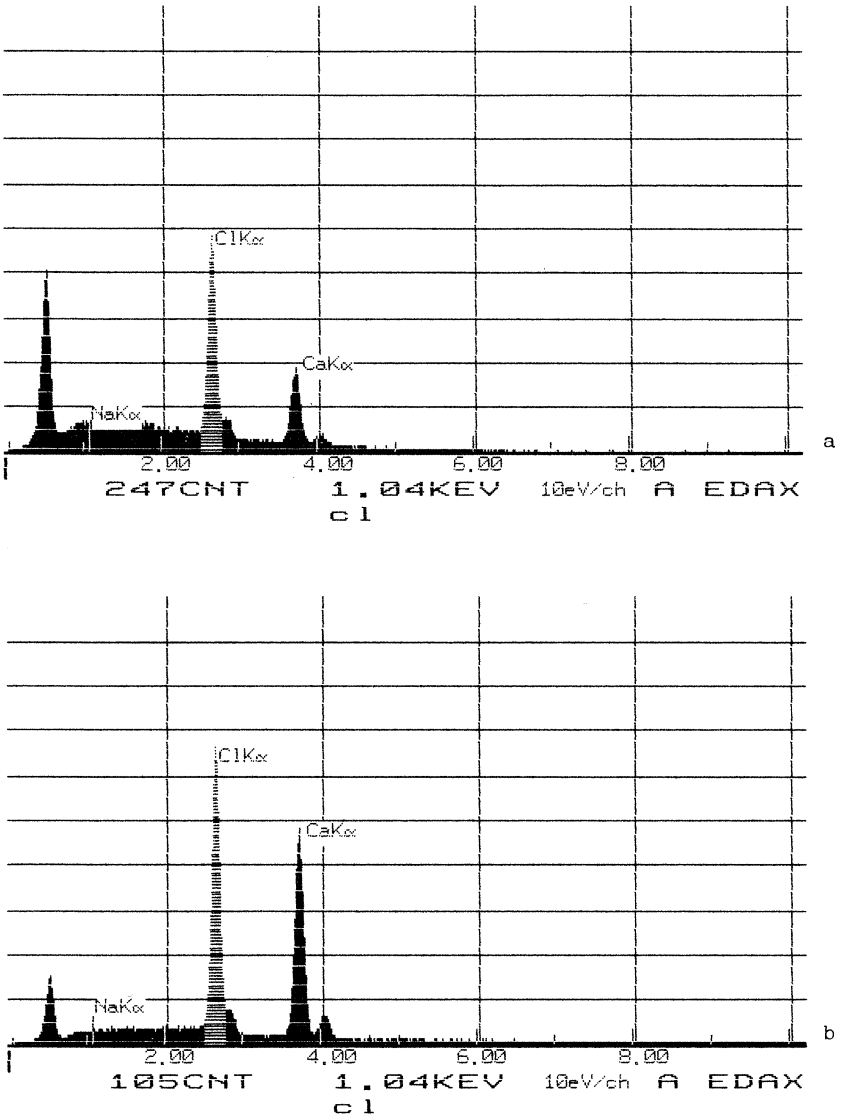

Fig. 6. Microanalysis chart corresponding to the chitosan (0.1%)–alginate beads: (a) surface; and (b) inner.

Alginate is known not to swell in acidic medium (Yotsuyanagi et al., 1991) and both types of beads remained intact during the 5 h test and no changes in shape were noted. At low pH, the presence of an abundance of positively charged ions reduces the electrostatic repulsion between the negatively charged alginate molecules and enhances the interaction between the negative charged alginate and the chitosan. In addition, at low pH, the non-swelling should reduce the matrix permeability and limit the drug diffusion.

The release profiles of DHEP from beads in pH 7.4 Sorensens phosphate (Fig. 9) shows that $\approx$ 40% of the incorporated DHEP is released in 5 h in both the Eudragit® L/alginate and 0.1% chitosan/alginate beads. The final percentage increased (60%) with an increase in the chitosan/alginate ratio. The release profiles of DHEP from the chitosan beads were dependent on the chitosan concentration. As the chitosan

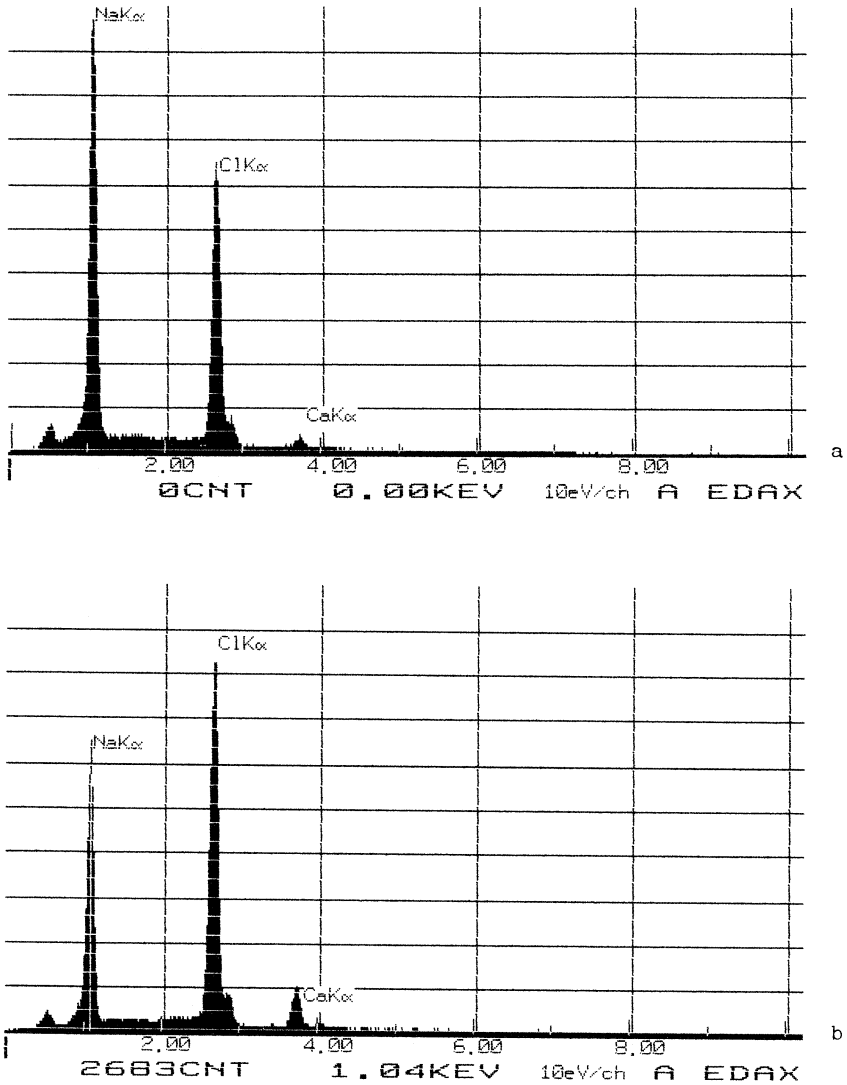

Fig. 7. Microanalysis chart corresponding to the chitosan (0.2%)–alginate beads: (a) Surface; and (b) inner.

concentration was increased, drug release was also increased. These unexpected results are in agreement with those reported by Polk et al., 1994 who observed an increase in permeability of albumin chitosan–alginate microcapsules when the chitosan concentration was also increased.

The release mechanism of the chitosan/alginate beads is related to the solubility of chitosan, which is poorly soluble in water. In acidic medium, protonation of the amine groups improves the solubility. The presence of negative charges due to the anionic nature of the alginate forms an interpolymeric complex which reduces drug release. At low pH, the interpolymeric complex exists in a gel form. When the beads were transferred to pH 7.4, the viscous complex previously formed swelled and the gel was slowly disintegrated, releasing the drug. The release rate is a function of the degree of cross-linking between both polymers (Fig. 10). Additionally, the similarity of the two saccharide structures offers greater possibilities for interaction between the opposite

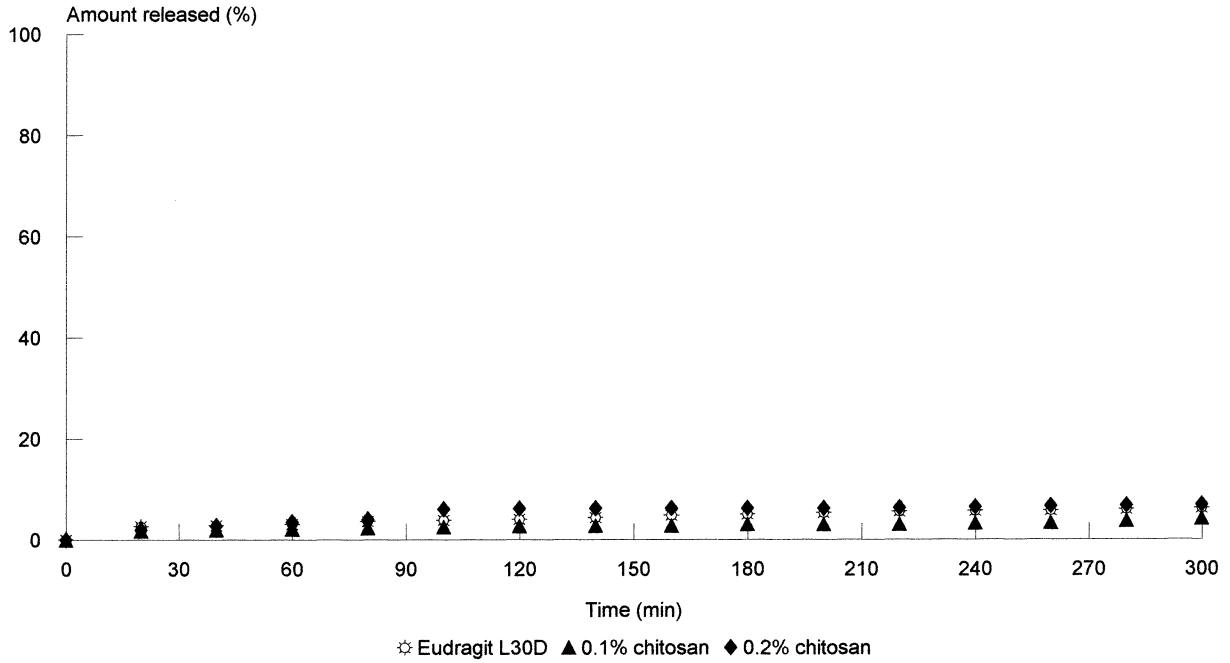

Fig. 8. Amount released of the beads at pH 1.1 in 0.1 N HCl.

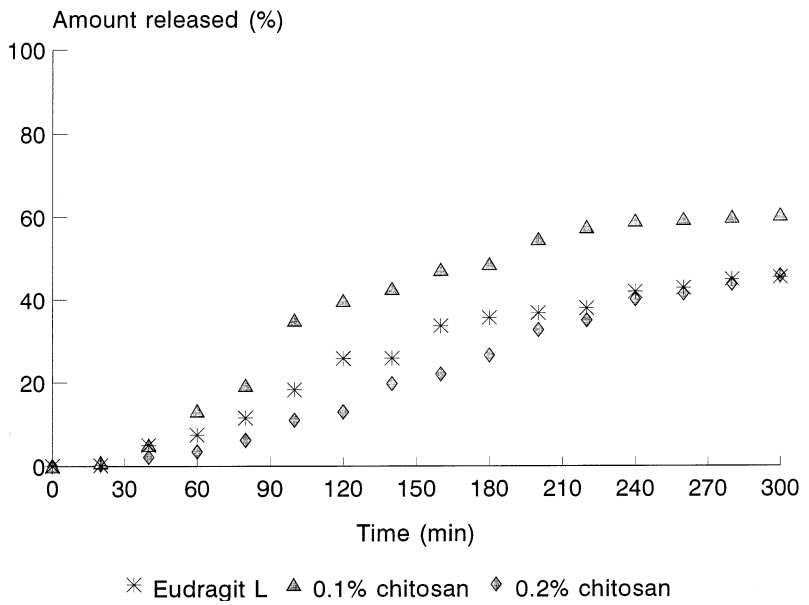

Fig. 9. Amount released of the beads at pH 7.4 Sorensens phosphate buffer.

charges of the two biopolymers. This controlled release process cannot be obtained when a pH sensitive material such as Eudragit® L30D is used because the release occurred immediately on changing the pH due to the dissolution of the Eudragit®.

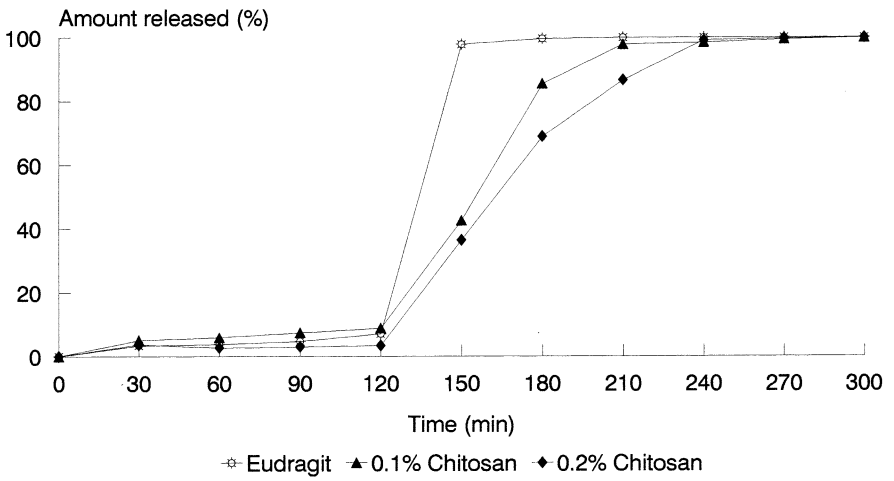

Fig. 10. Amount released of the beads at pH 1.1 (2 h) and pH 7.4 Sorensens phosphate (3 h).

In conclusion, the use of chitosan permits an improvement in the stability of alginate beads and a controlled release system can be produced by modifying the chitosan concentration. The behaviour of the beads at low pH and the high release at high pH are of great interest for the delivery of drugs such as NSAIDs and peptides into the small intestine. Further investigations are being carried out in order to study the influence of other variables such as molecular weight of chitosan, chitosan/alginate ratio and microencapsulation technique. Further studies will be carried out using NMR spectroscopy in order to investigate the nature of interaction between DHEP and chitosan.

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